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TOPIC 06 – Oxydative stress, aging

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Statins treatment induces apoptosis in vivo by increasing mitochondrial ROS in skeletal muscles

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Although statins are the most widely used cholesterol-lowering agents for prevention of obstructive cardiovascular events, there is a risk of myopathy occurring in patients taking these drugs. The underlying mechanisms remain unclear. The purpose of this study was to determine the effects of statins on oxidative stress and cell death in humans, rat and cell models.

We showed that statins increased mitochondrial H_2O_2 production and Bax/Bcl-2 ratio in human deltoid biopsies. Furthermore, atorvastatin treatment during two weeks at 10mg/kg/day increased the pro-apoptotic Bax mRNA relative expression level and increased the Bax/Bcl-2 immunostaining ratio in the plantaris muscle of rats (glycolytic muscle) but not in the soleus muscle (oxidative muscle). The TUNEL staining confirmed this increase of apoptosis only in plantaris. The exposure of L6 cells with atorvastatin increased the mitochondrial superoxide anion production, and decreased ATP production levels. The co- incubation with the antioxidant molecule N-acetylcystein (NAC) prevented these deleterious effects.

Altogether we showed that statins, by increasing mitochondrial oxidative stress in skeletal muscle, triggered the activation of the mitochondrial apoptotic pathway. These results suggest that the appearance of a statin-induced myopathy, observed in about 5% of patients treated with these drugs, could be partially explained by this ROS-induced apoptotic pathway. Future research could be concentrated on developing treatments with the ROS scavengers against statin-induced oxidative stress in order to inhibit apoptosis.

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Ageing-associated impairment of cardiac mitochondrial bioenergetics in healthy Sprague Dawley rats

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Background: Attenuation of mitochondrial bioenergetics is postulated to play a major role in the process leading to cellular ageing; yet conflicting. Results are reported on individual components of mitochondrial function according to different species and tissues. The present study was purported to characterize and compare oxidative phosphorylation, mitochondrial membrane potential ($\Delta\psi$), mitochondrial permeability transition pore (mPTP) sensitivity to calcium overload, and reactive oxygen species (ROS) production in rat heart mitochondria isolated from old (20-24 months) as compared to adult (4-6 months) Sprague-Dawley rats.

Methods: For the respirometry and $\Delta\psi$ measurements an Oxygraph-2k (Oroboros Instruments, Austria) equipped with an ion selective electrode filled with tetraphenyl-phosphonium was used. ROS production and the amount of total mitochondrial Ca^{2+} retained prior to mPTP opening were measured by spectrofluorimetry.

Results: Our results showed an important decline of $\Delta\psi$ and of all mitochondrial bioenergetic parameters together with an increased rate of ROS production in mitochondria from old vs adult group. In the old group, the mitochondrial sensitivity to Ca2+-induced mPTP opening was increased and the protective effect of CsA on mPTP opening was significantly reduced.

Conclusions: Our data suggest an age-dependent impairment of mitochondrial function in "healthy" Sprague-Dawley rats.

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Role of Gadd45/p38 MAPK pathway in stressed cardiomyocytes

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Ventricular remodelling is accompanied and influenced by perturbations in oxidative stress status and MAPK signalling. The Growth arrest and DNA-damage-inducible 45 (Gadd45) proteins are small acidic proteins involved in DNA repair and modulation of MAPK activities. However, their role in determining cell death in the heart is underexplored. Our aim is to explore the p38 MAPK dependant, and independent, roles of Gadd45 γ in myocardial injury response.

We examine our hypotheses using our existing in vitro models of isolated cardiac myocytes exposed or not to H2O2. Intracellular production of H2O2 was obtained using a recombinant MAO-A adenovirus. To study the effect of Gadd45 γ we used two complementary techniques: silencing RNA approach to knockdown Gadd45 γ gene and an adenoviral vector strategy to overexpress Gadd45 γ.

Free radical production induced apoptosis *via* p38 MAPK activation. Our results support the implication of Gadd45 γ in p38 MAPK phosphorylation during H2O2 exposition. Cardiomyocytes were exposed or not to H2O2 in a dose and a time dependent manner (0-88 µM from 0-120 min). We first observed a dose dependant increased in p38 MAPK phosphorylation and Gadd45 γ expression level after 15 min of exposure. The siRNA strategy was able to significantly reduce Gadd45 γ expression and p38 MAPK phosphorylation levels. We then exposed cardiomyocytes to 88 µM of H2O2 for up to 120 min. The peak of p38 phosphorylation and Gadd45 γ expression were at 15 min of H2O2 exposure and slightly decreased after 2h. The ratio of BAX/BCL2 is increasing during H2O2 exposure. The knockdown of Gadd45 expression was associated with a decreased in p38 phosphorylation and BAX/BCL2 ratio. In addition we observed that H2O2 production by the MAO-A is responsible to Gadd45 γ overexpression, we surexpressed MAO-A in cardiomyocytes and we treated with an antioxidant (Trolox, 1 mmol/L). The trolox treatment is able to reduce the Gadd45 γ overexpression. These preliminary results confirm the importance of Gadd45 γ to promote p38 phosphorylation and apoptosis marker. This project will demonstrate the importance of Gadd45 γ/p38 MAPK complex in the development and persistence of heart failure, particularly in the balance of the cell death process.

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Reduced GTP-cyclohydrolase activity in hph-1 mice selectively induced alterations of microvessels endothelial function with age

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Endothelial NO synthase (NOS3) activity depends on tetrahydrobiopterin (BH_4) bioavailability. BH_4 is synthesized by a cascade of enzymatic pathways in which the GTP-cyclohydrolase I (GTP-CH) is the rate-limiting enzyme. Hyperphenylalaninemic mutant mice (hph-1), characterized by reduced GTP-CH expression and activity provides a good model to investigate the role of BH_4 in the endothelial function of large conduit and small resistance vessels. The aim of this study was to compare

the consequences of its deficiency in these different vessels in young adult (12wo) and old (35 to 45wo) mice. In endothelial cells from 12-35wo hph-1 mice aorta, BH_4 content was 3-6 times lower than in wild-type (WT) mice. In parallel, cyclic GMP was reduced and superoxide anion production increased, indicating that NOS3 was uncoupled. Surprisingly, no functional consequences were observed in conduit vessels as the endothelium-dependent relaxations to acetylcholine (ACh) were similar in hph-1 and WT mice. The function of coronary arteries (large and microvessels) was then studied using isolated Langendorf heart technique. Basal coronary blood flow as well as reactive hyperaemia subsequent to a brief period of ischemia (partly NO-mediated), were unchanged in heart of 45wo hph-1 compared to WT. In 250 µM diameter-perfused mesenteric artery (resistance vessels), flow-mediated dilation was similar in the 2 groups of 12wo mice and decreased by 31% in 35wo hph-1 mice. Moreover, cutaneous microcirculation of these aged mice, measured *in vivo* by laser-Doppler technic, showed a 67% decreased response to ACh compared to WT mice. These results show that endothelium-dependent vasodilation of conduit vessels from hph-1 mice is maintained, whatever the age, despite uncoupled NOS3, suggesting the involvement of compensatory mechanisms. By contrast, alteration of endothelial function appears with age in microvessels revealing the key role of GTP-CH activity in the endothelial function of resistance vessels.

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Apoptosis promotion is involved in the cell death and the dysfunction of diving vascular endothelial cell during in vitro air diving simulation

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Influence on physiological function of vascular endothelium during dive plays a key role in diving-related injuries, like decompression sickness (DCS). In this study, through fluorescent real time monitoring the impact of *in vitro* diving simulation (8 ATA) on the viability of arterial endothelial cell was investigated and the ratio of apoptosis of diving endothelial cell was examined.

An *in vitro* diving system was constructed to achieve real time monitoring of cell activity under fluorescent microscopy during simulated dives. Endothelial cells were isolated from calf aorta and loaded with sapphire windows into the system before diving simulation. Fluorescent dyes, including calcein-AM, propidium iodide (PI) and annexin-V, were used to detect viability, death and apoptosis of diving cells, respectively.

Compared to non-dive group, the ratio of cell death and the percent of cell death of diving endothelial cells showed time-dependent increases during 45 min-diving simulation. Higher percent of apoptosis was presented in diving endothelial cells through the air diving simulation.

As a conclusion, viability of diving endothelial cell is impacted time-dependently during air diving simulation at 8 ATA. Apoptosis is induced in the endothelial cell through its air diving simulation and impacts the activity and the function of endothelial cell. For the further step, intracellular content of reactive oxygen species (ROS) will be evaluated as well as the function of mitochondrial respiration chain inside diving endothelial cell will be examined, in order to further investigate the influence on the mitochondrial activity caused by air diving simulation and the role of ROS in the induction of apoptosis in diving endothelial cells.

Keywords: Diving; endothelial cell; apoptosis; ROS; mitochondria

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Protein Tyrosine Phosphatase-1B deletion in mice improves cardiac and endothelial functions during aging

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Cardiovascular aging is associated with left ventricular (LV) hypertrophy, myocardial fibrosis and endothelial dysfunction, all contributing to heart failure establishment. Our laboratory showed that Protein Tyrosine Phosphatase 1B (PTP1B) deletion confers myocardial and endothelial protection during chronic heart failure. However, whether PTP1B deletion could slow aging-associated cardiovascular damages remains unknown.

We assessed LV function by echocardiography in PTP1B deficient (PTP1B-/-) and wild-type (WT) mice aged of 3, 12, 15 and 18 months. At 18 months, LV function was assessed by invasive pressure-volume curves that measure LV end-systolic and LV end-diastolic pressure-volume relationships (LVESPVR and LVEDPVR). We also evaluated mesenteric flow-mediated dilatation (FMD) by arteriography and coronary function in Mulvany-wire-myograph. Finally, histological analyses of heart remodeling were performed.

Echocardiographic analyses showed that the aging-dependent decrease of LV fractional shortening was lesser in PTP1B-/- mice, compared to WT (18 months vs 3 months: WT: -54%; PTP1B-/-: -29%, $p<0.001$). LV diastolic dysfunction evidenced by the modification of the trans-mitral Doppler E/A ratio was significantly improved in PTP1B-/- mice. At 18 months, PTP1B-/- mice showed a higher LVESPVR (WT vs PTP1B-/-: 13.9±0.9 vs 18.4±1.6 mmHg/RVU, $p<0.05$) and a lower LVEDPVR (WT vs PTP1B-/-: 5.1±0.8 vs 1.2±0.3 mmHg/RVU, $p<0.01$), highlighting the improvement of both LV systolic and diastolic functions. Moreover, PTP1B deletion partially restored FMD in mesenteric arteries (% FMD at 200µL/min; WT vs PTP1B-/-: -0.4±2.1 vs 7.4±2.6, $p<0.05$) and slightly improved coronary artery relaxations in response to insulin (% relaxation at 10-6M: WT vs PTP1B-/- 15±4 vs 24±3). Finally, cardiac hypertrophy, capillary density and fibrosis were significantly reduced in PTP1B-/- mice.

Taken together, these results show that PTP1B deletion improved cardiac and endothelial functions during aging.